

Profile of *Parkia speciosa* Hassk Metabolites Extracted with SFE using FTIR- PCA Method

Nik Norulaini Nik Abd. Rahman,^{b,*} Salman Zhari,^a Md. Zaidul Islam Sarker,^{c,*} Sahena Ferdosh,^d
Mohd Azizi Che Yunus^a and Mohd Omar Abd Kadir^d

^aFaculty of Chemical Engineering, Universiti Teknologi Malaysia, Skudai, Johor Bharu, Malaysia

^bSchool of Distant Education, Universiti Sains Malaysia, 11800 Minden, Penang Malaysia

^cDepartment of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan Campus, Badar Indera Mahkota, 25200, Kuantan, Pahang D/M, Malaysia

^dSchool of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang Malaysia

Received February 22, 2011; Accepted July 4, 2011; Published Online August 5, 2011

A rapid identification, classification and discrimination tool, using Fourier Transform Infrared (FTIR) spectroscopy combined with Principal Component Analysis (PCA), was developed and applied to determine the profile of the Supercritical Fluid Extraction (SFE) of *Parkia speciosa* seeds under various temperature and pressure conditions (313, 323, 333, 343, 353 and 363 K and 20.68, 27.58, 34.47, 41.37, 48.26, and 55.16 MPa). The separation and identification of the compounds was carried out by Gas Chromatography coupled with Time of Flight Mass Spectrometry (GC/TOF-MS). This technique has made it possible to detect the variability obtained under different SFE conditions and the separation of different chemical compounds in *P. speciosa* seeds. The FTIR-PCA results were verified by GC/TOF-MS, and the FTIR-PCA method successfully identified the unsaturated carboxylic acids with the highest percentage area under the different conditions.

Keywords: SC-CO₂ extraction; FTIR-PCA identification; *Parkia speciosa* Hassk seeds; Fingerprinting; Retrograde vaporisation.

INTRODUCTION

Parkia speciosa Hassk is a tropical leguminous tree in the family of *Leguminosae* found in most Southeast Asian countries. The seeds are used as food, either cooked or raw, due to their high nutritional value. The seeds are known to have important chemical and medicinal compounds, such as several cyclic polysulphides, which are used for the treatment of antibacterial activity in kidney, ureter and urinary bladder infections,¹ and thiazolidine-4-carboxylic acid, which is used for its anticancer activity.² The seeds also have a hypoglycaemic effect, due to the synergistic action of 8-sitosterol and stigmasterol.³

The important chemical and medicinal compounds in *P. speciosa* have not been systematically identified. A gas chromatograph can be used to separate the components of a mixture; and the fractions containing single components can be directed into an FTIR spectrometer, to provide the infrared spectrum of the sample. Initially, the use of infrared

(IR) spectroscopic methods was restricted to the structural elucidation of isolated compounds from herbal matrices. It was also found to be useful in phytochemical studies as a fingerprinting device, for comparing natural and synthetic samples.⁴ Due to the inherent complexity of the IR spectrum, the actual interpretation may be difficult and the operation requires much experience. Indeed, slight differences in the spectra within the same plant species may not be obvious and are generally not visible to the naked eye. Thus, the application of IR spectroscopy in herbal analysis is still very limited as compared to its application in other areas (food and beverage industry, microbiology, pharmaceuticals, etc).

Principal Component Analysis (PCA), an unsupervised chemometric pattern recognition technique, is frequently used in handling multivariate data without prior knowledge of the samples studied.⁵ The main advantage of chemometrics is its ability to analyse very complex multi-

* Corresponding author. Tel: +604-6532108; +609-5716687; Fax: +604-6585435; +609-5716775; E-mail: norulain@usm.my (Nik Norulaini Nik Abd Rahman), zaidul@iium.edu.my (Md. Zaidul Islam Sarker)

variate datasets, such as those from GC-MS, DAD-HPLC, NMR, FTIR and NIR, involving overlapping peaks, very noisy chromatograms, thousands of samples and very similar profiles, which cannot be handled in a conventional way in environmental assessment, natural products, food or forensic analysis.⁶ Chemometric methods can be applied to extract information from datasets mainly for signal resolution, classification, calibration and experimental design. Many chemometric methods can be used for these purposes; however, PCA is the most commonly used method. PCA associated with FTIR spectroscopy has been shown to be an important tool for the rapid identification and classification of data and has been applied to the analysis of infrared spectra in the fields of medicine, biology and forensic science.⁷

Supercritical fluid extraction (SFE) using carbon dioxide is an environmentally friendly and efficient extraction technique⁸ for solid materials and has been extensively studied for the separation of active compounds from natural products. Due to its good solvent properties, CO₂ remains the most commonly used fluid for the extraction of non-polar components such as hydrocarbons.⁹ Analysing SFE data by applying PCA in analysis is still very limited, with the exception of a study reported by Yang et al.¹⁰ on the application of SFE/FT-IR/PCA in paper analysis. The researchers found that the significance of applying PCA is, it enables to rapidly discriminate between extractable compounds that are indigenous and non-indigenous compounds, based on their infrared spectra. The method was applied with on-line supercritical fluid extraction, capillary supercritical fluid chromatography and FTIR. They successfully detected the presence of a drug, lysergic acid diethylamide (LSD), on paper by applying this technique. In this work, FTIR spectroscopy was applied as a quantitative method to study the effects of the SFE condition on the functional group. The objective of this study is then to use Fourier Transform Infrared (FTIR) spectroscopy in tandem with Principal Component Analysis (PCA), as a rapid identification, classification and discrimination tool, to determine the profile of the Supercritical Fluid Extraction (SFE) of *Parkia speciosa* seeds under extraction conditions.

MATERIALS AND METHODS

Sample Preparation

P. speciosa seeds were obtained locally from Selama, Perak, Malaysia. The seeds were separated from the pods,

soaked in water overnight to remove the testae and then dried in an oven at 45 °C until reaching a constant weight.¹¹ The dried seeds were then ground, using a lab blender (Waring Laboratory, USA), and the particle size distributions were determined by sieve analysis, with a Vibrator Sieve Shaker (Retsch, Germany), and stored in a refrigerator at -20 °C in a sealed container.

Supercritical Fluid Extraction (SFE) System

Supercritical fluid extractions were performed using a system consisting of a CO₂ gas cylinder (MOX Gases Bhd, Selangor, Malaysia), a chiller (Yih Der BL-730), and an ISCO SFE System (ISCO Inc., Lincoln, NE, USA) consisting of a supercritical fluid extractor (SFX 220), a controller (SFX 200), a syringe pump (model 100DX) and a restrictor temperature controller associated with two coaxially heated capillary restrictors.

Method of Extraction

For each extraction process, a 10-mL sample cartridge was filled with 1.5 g of dried seeds of *P. speciosa*. Static extraction was performed for 5 min before the pump was turned on, to stabilise the temperature, and for another 5 min after the set pressure was reached, before the exit valve was opened. A fraction of the extract was collected every 5 min, and the extraction was carried out for 50 min. The yield was determined as gram extract per gram sample.

Soxhlet extraction of *P. speciosa* seeds using hexane

Soxhlet extraction on 20.0 g of ground seeds with particle sizes < 250 µm was performed for 2 h. After the extraction process, the solvent was removed using a rotary evaporator. The extract was then dried to remove the remaining solvent.

ATR-FTIR Spectroscopy

All the FTIR spectra were collected with a Nicolet Nexus Spectrometer (ThermoNicolet) coupled with a temperature-regulated DTGS (deuterated tri-glycine sulphate) detector. The FTIR spectra were recorded in the mid-IR region, 4000-675 cm⁻¹, at a resolution of 4 cm⁻¹ with 32 scans.

The raw data from the experiment with an extension file of (.spa) were copied to Spectrum v3.02 (Perkin Elmer) software and saved as an ASCII file for further analysis. The percentage transmittance data were substituted for the absorbance data. Baseline correction, spectrum smoothing and normalisation were carried out. The baseline of the

spectrometer was removed by auto-correcting the spectrum, while normalisation of the absorbance spectra to the most intense peak removes the differences between the spectra due to different amounts of sample or path length variation.¹²

Chemometrics analysis using principal component analysis (PCA)

The acquired ASCII extension file spectra were imported into the multivariate statistics program Unscrambler 9.0 (CAMO, India). PCA was carried out using 1712 points (normalised absorbance) within the spectral region 4000–700 cm⁻¹. Leverage correction was used as the validation method while weights were fixed at 1.0. A model size of 10 principal components (PCs) was used. The grouping of *P. speciosa* from 36 different SFE extraction conditions and a hexane extraction were visualised in the two-dimensional score plot. The structural difference in the dataset from the PC scores was revealed by the loading plot.

Chromatographic analysis by GC-TOFMS

A sample of 5 µL was taken into a vial, mixed with 995 µL hexane (1:199) and homogenised using a vortex (IKA-Labortechnik STAUFEN, German) for 2 min. The sample was then filtered into a 2-mL collection vial for chromatographic analysis. The gas chromatography analysis was carried out using a system consisting of an Agilent Technologies 6890N Series and 7683 Series auto sampler injector with controller, coupled with a LEGO Pegasus III reflection Time of Flight (TOF) Mass Spectrometer with element impact ionisation, equipped with a Chrom-TOF mass data analysis system.

For the mass spectrometer settings, the mass range chosen was 35–550 atomic mass unit (amu), the spectra scan rate was 20 spectra per second and the ion source temperature was set to 250 °C. The identification of the oil compounds was performed by similarity searches in the NIST (National Institute for Standard and Technology) and 1998 Mass Spectral Wiley Database libraries.

RESULTS AND DISCUSSION

Parkia speciosa seed extracts

The SF extracts of *P. speciosa* were light to dark green in colour, and their aroma was similar to that of other hexane extracts. The global extraction yield for hexane extract was 14.2% while the global extraction yield for SF ex-

tracts ranged between 5.57–23.7%, depending on the temperature and pressure of the extraction condition, as shown in Table 1. The highest extracted yield (23.7%) was achieved at high pressure and high temperature (55.16 MPa, 363 K). Interestingly, the lowest extracted yield (5.57%) was collected at high temperature as well, but at lower pressure (20.68 MPa, 363 K). This interesting behaviour is referred to as *retrograde* vaporisation. In supercritical fluid extraction, the use of varying temperature and pressure and the addition of co solvents are ways to optimize the extraction efficiency to overcome the low yield and selectivity. The phenomenon of retrograde vaporiza-

Table 1. Yield of *P. speciosa* seeds at different pressure and different temperature

Pressure (MPa)	Temperature (K)	Experimental Code	Extracted yield (g)	Extracted yield (%)
20.68	313	3P4T	0.1888	12.41
	323	3P5T	0.1803	11.83
	333	3P6T	0.1657	10.94
	343	3P7T	0.1387	9.12
	353	3P8T	0.1031	6.90
	363	3P9T	0.0838	5.57
27.58	313	4P4T	0.2436	16.22
	323	4P5T	0.2549	17.01
	333	4P6T	0.2636	17.59
	343	4P7T	0.2429	16.25
	353	4P8T	0.2202	14.75
	363	4P9T	0.2003	13.30
34.47	313	5P4T	0.2896	19.18
	323	5P5T	0.3012	19.48
	333	5P6T	0.3172	20.80
	343	5P7T	0.3161	20.88
	353	5P8T	0.3108	20.71
	363	5P9T	0.2981	19.72
41.37	313	6P4T	0.3061	20.50
	323	6P5T	0.3223	21.56
	333	6P6T	0.3387	22.31
	343	6P7T	0.3438	22.74
	353	6P8T	0.3455	22.92
	363	6P9T	0.3432	22.98
48.26	313	7P4T	0.3197	21.29
	323	7P5T	0.3317	22.01
	333	7P6T	0.3407	22.69
	343	7P7T	0.3478	22.75
	353	7P8T	0.3530	23.38
	363	7P9T	0.3540	23.37
55.16	313	8P4T	0.3214	21.27
	323	8P5T	0.3399	22.54
	333	8P6T	0.3477	23.17
	343	8P7T	0.3545	23.58
	353	8P8T	0.3563	23.64
	363	8P9T	0.3568	23.70

tion can be used to explain the increase in the difference in solubility of the components to be separated, without much affecting the yield. The retrograde vaporization is the unexpected solubility behavior under high temperature and pressure when the chemical compositions approach nearly pure volatile component. At low pressure the solvent power of CO₂ decreases with rising temperature; whereas at high pressures the solubility increases proportionally. The solubility-temperature relationship comes about because density decreases dramatically with an increase in temperature at low pressure; whereas at higher pressure, changes in temperature have much less effect on density. Thus density, not pressure, to a first approximation is proportional to the solvent power of the SFE.¹³

The results from the experiments show that increasing the pressure from 20.68 to 55.16 MPa at constant temperature increases the extracted yield. This may be attributed to the increased density and solvating power due to greater attractive forces between the supercritical fluid (SF) and the solute molecules.¹⁴ At lower pressure and temperature, supercritical fluid extraction (SFE) yields were poor, as compared to the hexane extractions. This indicates that the solvent strength of carbon dioxide at higher pressure was sufficient to replace hexane as a solvent for extraction. One advantage of SFE over solvent extraction is the selective extraction and fractionation of desired compounds made possible by altering the density.

Qualitative analysis of SC-CO₂ extract of *P. speciosa* by FTIR-PCA

Figure 1 shows FTIR spectra of four different interaction conditions of supercritical fluid (SF) and hexane extracted *P. speciosa*. The spectra consist of different groups of absorption bands at wave numbers ranging from 4000-

700 cm⁻¹. The bands at 3412-3391 cm⁻¹ (O-H stretching) and 1649-1648 cm⁻¹ (H₂O bending) are characteristics of water. These bands are only identified in the high pressure extracts. Although this is quite surprising, the spectra of extracts at high pressure are not affected and still provide very meaningful information. A band appearing at 3008.45 cm⁻¹ was assigned to the =CH asymmetric stretching (vas) of cis fatty acids. The C-H stretching of methylene (vasym) and methyl (vsym) bands were identified at 2923.60 cm⁻¹ and 2856-2855 cm⁻¹, respectively.

The region of 1800-700 cm⁻¹ is the fingerprint region of the individual bonds of the functional groups. The class identification, corresponding to the peaks/bands in this region, was made using PSU (Possible Structural Unit) tools in the Spectrum v3.02 software.

The sharp bands at 1747-1746 cm⁻¹ are assigned to C=O stretching vibrations and may be characterised by the presence of high amounts of carboxylic acid in the complex mixture of *P. speciosa* extracts. Under the SF extracted conditions of 20.68 MPa and 353 K, a small peak unique to the extract carried out under low pressure and high temperature conditions was identified at 1712.50 cm⁻¹, corresponding to class 3 carbonyl compounds. These class 3 carbonyl compounds such as aldehydes, ketones, esters, and amides have a second site of reactivity which is the alpha-carbon. Reactions at the α -carbon play an important role in several biological processes such as glycolysis, fatty acid synthesis, and the process that causes meat from older animals to be tougher than meat from younger ones.

A sharp peak at 1470-1460 cm⁻¹, a small peak at 1379-1376 cm⁻¹, a small peak at 1240-1236 cm⁻¹ and a small sharp peak identified at 726-724 cm⁻¹ may be assigned to sulphur-containing compounds. The broad sharp peak at 1167-1164 cm⁻¹ may be assigned to polysaccharides or sulphur compounds. By visual observation, there are no significant differences between the characteristic absorption bands of samples extracted with SF under different conditions and the hexane extracted sample. However, the intensities of certain wavelengths differ from each other, particularly within the fingerprint region (1800-700 cm⁻¹), as presented in Fig. 1.

As the differences between the spectra are generally not visible to the naked eye, it is more practical to incorporate statistical methods in order to interpret the results obtained from spectroscopic analysis. Since the discrimination of different conditions of extract based on the slight differences among particular bands is too subjective, the

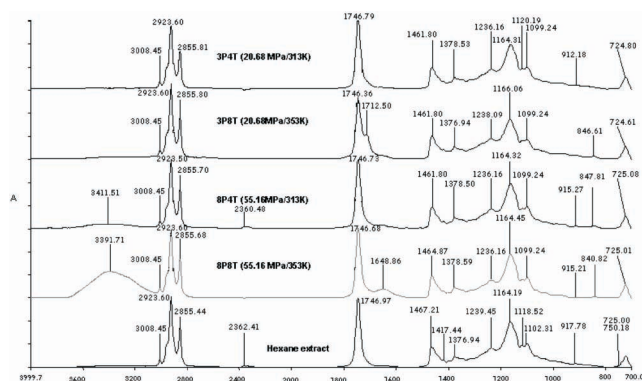


Fig. 1. FTIR spectra of *P. Speciosa* extract showing absorbance in the region of 4000-700 cm⁻¹.

results may vary between analysts.¹⁵ Hence, a more reliable method for discrimination of samples, by incorporating chemometric analysis in interpreting the resultant spectra, was applied.

The principal component analysis (PCA) fulfils two objectives; first, it transforms the data into a more relevant co-ordinate system (which lies directly in the centre of the data points), and second, it performs a dimensionality reduction (using only the first principal components, which reflect the structure in the data).¹⁶

The PCA of 37 objects (36 from SC-CO₂ and 1 from hexane extraction) was carried out and indicates that the total variance of the two components is 93% (PC₁ = 80% and PC₂ = 13%) and that the data of the soxhlet hexane sample is well separated from that of the SC-CO₂ samples as visualized in the 2-D score plots (Fig. 2). Thus, these components were sufficient to provide effective clustering¹⁷ of the types of samples. Meanwhile, the corresponding 1-D loading plots, of the 37 *P. speciosa* extract samples, is shown in Fig. 3. In spectroscopy, the 1-D loading plots are often a great advantage, as they are very useful for the assignment of diagnostic spectral bands. This loading plots indeed shows great similarity to the spectrum. The loadings that belong to PC1 can partially related to the presence of water while PC2 belongs to other compounds. Variables with a high degree of systematic variation typically have large absolute variances, and consequently large loadings. In a 2-vector score plot, sample 7P7T, 8P8T and 8P9T lies far away from the origin. This means that sample 7P7T, 8P8T and 8P9T has high value of water. If we take into account

what we know about PC 1, then higher pressure conditions (positive scores) seems to give extracts with high water contents, while lower pressure conditions results in extracts which are free from water. Now it will also be clear that PC2 can be interpreted as the levels of most major compounds estimated by FTIR intensity. The intensity levels of most compounds in *P. speciosa* hexane extract was relatively high compared to supercritical fluid extracted *P. speciosa*. Vesper and Nitz¹⁸ compared the use of supercritical CO₂ to the use of hexane in the extraction of paprika and found that hexane had slightly higher carotenoid content while extracts from SFE has higher water content.

The PCA of 36 samples of SFE was carried out again to investigate their distribution under different SFE temperature and pressure conditions. The total variance of the two components was 97% (PC₁ = 91% and PC₂ = 6%) as shown in Fig. 4. The corresponding 1-D loading plot, shown in Fig. 5 shows that the loading belonging to PC1 may be partially related to the presence of water, while that of PC2 relates to other compounds. Thus, higher pressure

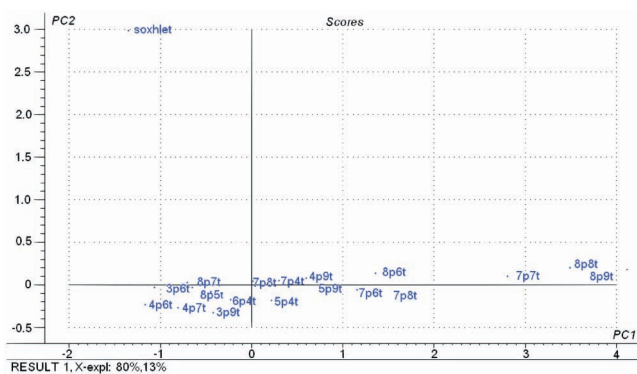


Fig. 2. 2-D score plots of FTIR spectra *P. speciosa* extracted by SFE and soxhlet with well separated data. The SFE plots are the experimental codes for the extractions under different pressure (p) and temperature (t) used. The variance of the two components are PC₁ = 80% and PC₂ = 13%.

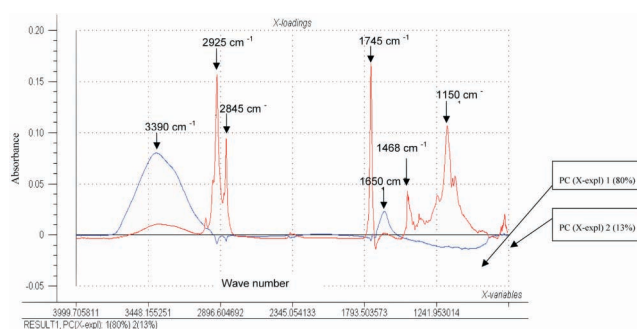


Fig. 3. Loading plots of FTIR spectra from *P. speciosa* extracted by SFE and soxhlet.

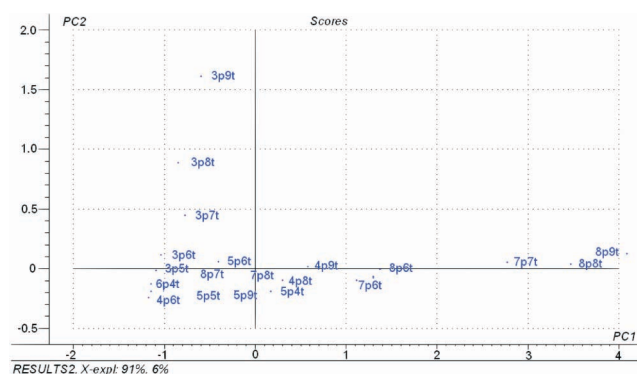


Fig. 4. 2-D score plots of FTIR spectra *P. speciosa* extracted by SFE under different temperature (t) and pressure (p) conditions expressed in experimental codes. The variance of the two components are PC₁ = 91% and PC₂ = 6%.

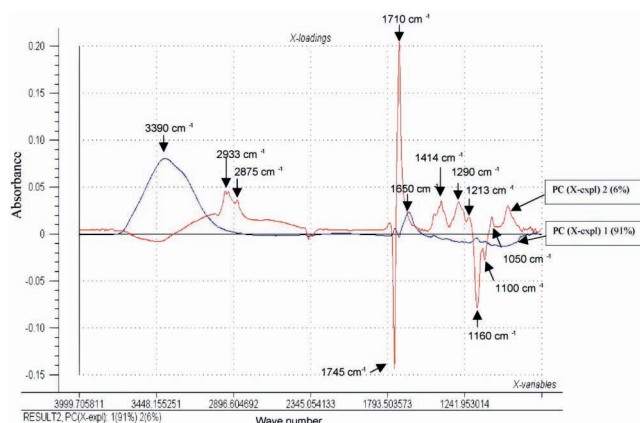


Fig. 5. Loading plots of FTIR spectra from *P. speciosa* extracted by SFE.

conditions (positive scores) produce extracts with high water content (at a pressure of 55.16 MPa and temperatures ranging from 313 to 363 K), and lower pressure results in water-free extracts. From the loading plots PC2 shows features characteristic of unsaturated carboxylic acids as estimated by FTIR intensity. PC2 features peaks at 2933, 2875, 1710, 1650, 1414, 1290, 1213, 1050 and 933 cm^{-1} typically involve compounds like oleic, linoleic and natural acid mixtures, while it is possible that the structure of the peaks at 1745, 1160 and 1100 cm^{-1} represents ester or ketone carbonyl compounds PC2.

GC-TOF-MS analysis

The number of components identified by GC-TOFMS analysis of *P. speciosa* seed SF extract shows an interesting result. Table 2 shows the list of components used to determine the difference between the compositions of the SF and hexane extracts. As is evident from the table, the supercritical extract obtained under all conditions shows a much higher area percentage of fatty acids and their esters. In contrast, the percentage area of phytosterols obtained from the hexane extracts is much higher than that of the SF extracts. Ab-Rahman et al.¹⁹ extracted α -tocopherols and squalene from the leaves of *Pandanus odoratus*; the yields of both compounds increased with increasing pressure but decreased with increasing temperature. The extraction of residual oil from screw-pressed palm fruit cake by Nik Norulaini et al.²⁰ extracted showed that more saturated fatty acids could be recovered at 50 °C and lower operating pressure of 13.7 MPa and that unsaturated fatty acids, such as linoleic and oleic acids, were retrieved at higher pressures such as 27.6 and 34.5 MPa.

The highest number of compounds were achieved at the smallest percentage of extracted oil yield. The samples that were extracted under high temperature conditions (353 K) were observed to have 34 and 30 components, whereas those at low temperature (313 K) consisted of 24 and 25 compounds at low pressure (22.68 MPa) and high pressure (55.16 MPa), respectively. Jaren-Galan et al.²¹ analysed the effects of temperature and pressure on the composition of paprika extract and found that the selectivity of CO_2 is higher at lower temperature (40 °C). Hexane extracts were identified as containing 43 components. The composition percentage is represented as the relative area (peak area relative to total peak area). In total, 77 compounds were identified based on the percentage of similarity and peak areas of more than 75 and 0.3%, respectively, in a 55-min analysis. In order to discriminate the solvent from the list of compounds, compounds from blank n-hexane were compared with the sample compounds, where the same compounds identified in both the blank and extracted samples were isolated. The compounds listed were collected after 200 s of spectroscopic analysis. Thiodipropionic acid and didodecyl ester were major components in three of the four extracted samples. The supercritical fluid extracts were characterised by a high percentage of fatty acids and their esters; 22.16 MPa/313 K (66.31%), 22.16 MPa/353 K (68.7%), 55.16 MPa/313 K (77.96%) and 55.16 MPa/353 K (76.82%). The main non-fatty acid components obtained under low pressure and low temperature (22.16 MPa/313 K) are 2-hexyl-1-Decanol (17.2%), p-sitosterol (3.6%), campesterol (2.41%) and stigmasterol methylether (2.29%). The main non-fatty acid compounds identified at the same pressure but at higher temperature (22.16 MPa/353 K) were p-sitosterol (5.03%), campesterol (3.64%) and stigmasterol (3.41%). At higher pressure and low temperature (55.16 MPa/313 K), p-sitosterol (3.63%) was the main non-fatty acid component identified, followed by campesterol (2.44%) and stigmasterol methylether (2.23%). Finally, at high temperature and high pressure (55.16 MPa/353 K) the extract was characterised by its high content of linoleic acid chloride (5.24%), p-sitosterol (5.41%), stigmasterol (3.21%) and campesterol (3.11%). Nik Norulaini et al.²² found that in supercritical carbon dioxide extraction of non-polar compounds from *Anastatica hierochuntica*, the pressure was the most significant parameter that affected the content of the compounds. They found that hexadecanoic and 9,12-octadecadienoic content decreased, while heneicosane and heptacosane increased with pres-

Table 2. Main component of *P. speciosa* supercritical fluid extract and soxhlet hexane extract (peak percentages are analysed by GC-TOFMS)

Compound	t_R (s)*	20.68 MPa/	20.68 MPa/	55.16 MPa/	55.16 MPa/	Soxhlet [#]
		313 K [#]	353 K [#]	313 K [#]	353 K [#]	
2-Decyn-1-ol	410.80					1.19
trans-2, trans-4-Decadienal	455.656			0.77	0.84	5.60
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	723.71			2.11		
Tetradecane	751.51					1.23
Undecanoic acid	1369.19		8.17		0.87	
Myristic acid	1372.19	0.83	12.24	0.36	1.15	
Palmitic acid	1373.35	7.14	12.24	5.62	6.06	1.85
Tetradecanal	1410.69				0.47	1.20
1,1-Octadecenoic acid, methyl ester	1493.02					0.48
Linolelaidic acid, methyl ester	1535.76				3.23	1.07
Stearolic acid	1544.21	6.52		2.84		
Hydnocarpic acid	1547.69		2.08			
Oleic Acid	1549.26	5.06		2.74		
Linoleic acid	1562.30	1.26	11.15		3.36	4.39
Elaidic acid	1566.55		3.18			
Stearic acid	1583.80	1.60	218	0.40	1.24	
Palmitic acid, butyl ester	1785.49	0.40		0.37	1.22	
Stearic acid, butyl ester	1792.55	0.75		0.68	2.39	
9,12-Octadecadienal	1939.00					1.24
Phthalic acid, mono(2-ethyhexyl) ester	1941.80		0.62			1.24
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-5-Nonyl-6-valeralactone	2064.50					1.42
Octadecanoic acid, 5-hydroxy, 5-lactone	2135.50					
Trifluoroacetic acid, n-tetradecyl ester	2140.74	1.47	0.90	1.77	2.04	5.06
Squalene	2141.34		4.44			
Campesterol	2184.45	2.41	1.10	2.44	0.43	1.08
Stigmasterol	2494.70	2.29	3.64	2.23	3.11	10.8
Stigmasterol methylether	2516.76	3.60	3.41	3.63	3.21	10.31
Stigmasterol	2518.65		0.97		5.41	1.32
Stigmastan-6,22-dien, 3,6-dedihydro-(3-Sitosterol)	2520.49		5.03			15.26
	2561.65		1.36			1.69
Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-	2564.70					
Trans-24-propylidenecholesterol)-4,4,6a,6b,8a,	2565.50	0.74	1.03	0.81	1.30	1.25
1,1,11,14b-Octamethyl	2575.70				0.38	1.50
12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	2614.56					2.85
1L,u4p,4eao,15,6,6a,6b,7,8,8a,9,10,11,						
2,6,10,14-Hexadecatetraen-1-ol, 3,7, 11,15-tetramethyl-,	2614.70					
a2c-Petraotpee, n3otriacn ascid, dodecyl ester	2761.34	1.73	1.10	6.65	13.96	
Thiodipropionic acid, didodecyl ester	2762.26	32.18	2.73	57.14	34.81	
2-Hexyl-1-Decanol	2763.16	17.2	0.74	5.24		2.33
Linoleic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	3208.61	6.56	3.07		1.22	1.40
Linoleic acid chloride	3470.15	0.97				
Arachidonic acid	3472.84	0.61				

Components are listed in order of elution on DB-5 column

* t_R is the retention time according to the GC method.[#] relative area = ratio of individual peak area to total peak area.

sure.

Abundance Ion Chromatography (AIC) illustrates the difference and variability found in the chemical profiles of

four samples under different SFE conditions and that of hexane extract. Although the chemical profiles of the four samples are considerably different, there are also some

common features in these samples. For example, thiodipropionic acid, didodecyl ester, β -sitosterol and campesterol were the main chemical compounds found in all the samples. Comparison of the composition of the SFE products with that of the hexane extracted oil reveals that higher percentages of phytosterols, β -sitosterol (15.26%), stigmasterol (10.31%) and campesterol (10.8%) were found in the hexane extract oil. As for the fatty acid compositions of the hexane extracts, the percentage is lower than the supercritical fluid extracted fatty acids at only 16.61%. The verification of data obtained from the chemometrics analysis by GC-TOFMS shows that at 20.68 MPa/353 K, the percentage area of unsaturated carboxylic acid (linoleic acid) was very high (11.15%) as compared to the other conditions. This verified the finding from chemometric analysis that the contents of unsaturated carboxylic acid at lower pressure and higher temperature were high.

CONCLUSIONS

The study of extraction from *P. speciosa* seeds shows that the highest percentage of extracted yields (23.7%) was obtained at 55.16 MPa and 363 K. Although the highest percentage yield was obtained at the highest temperature, the same high temperature but at the lowest pressure (20.68 MPa, 363 K), generated low yield at only 5.57%. These phenomena may result from the competing effect between the CO₂ density and analyte volatility, also known as retrograde vaporisation behaviour. The extractability of the analyte shows that the CO₂ density effects predominate up to a crossover pressure of 34.47 MPa. At pressures higher than 34.47 MPa, the volatility effects predominate, resulting in an increase in extracted yield (20.5% to 23.7%), although the CO₂ density decreases with increasing temperature at constant pressure.

The FTIR-PCA analysis of the spectra of SC-CO₂ extracts shows that the results obtained at low pressure (20.68 MPa) and higher temperature could be well discriminated from other samples. From the analysis of loadings in PCA, the samples could be distinguished by the higher contents of unsaturated carboxylic acid in the low pressure samples. The increase in temperature at constant low pressure resulted in higher contents of unsaturated carboxylic acid. Further analysis by GC-TOFMS verified these results. The score plots of PCA from the 36 samples further discriminated the high pressure samples (48.26 MPa and 55.16 MPa) from the other samples. Loading analysis demonstrated that these samples contain high moisture content, in

accordance to the water peak identified in the PC. Furthermore, the hexane extracted samples were almost free of water.

ACKNOWLEDGEMENT

The research facilities and financial help from MOSTI and Universiti Sains Malaysia (USM) are gratefully acknowledged.

REFERENCES

- Gmelin, R.; Susilo, R.; Fenwick, G. R. *Phytochemistry* **1981**, *20*, 2521-2523.
- Suvachittanont, W.; Kurashima, Y.; Esumi H.; Tsuda, M. *Food Chem.* **1996**, *55*, 359-363.
- Fathaiya, J.; Suhaila, M. M.; Nordin, L. *Food Chem.* **1994**, *49*, 339-345.
- Harbone, J. B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 3rd ed.; Chapman & Hall: London, 1998, pp 7-29.
- Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 4th ed.; Prentice Hall: New York, 2000, pp 217-220.
- Sukri, H. *Buletin Kualiti dan Teknikal* **2005**, *6*(1), 65-69.
- Razic, S.; Onjia, A.; Dogo, S.; Slavkovic, L.; Popovic, A. *Talanta* **2005**, *67*, 233-239.
- Goncalves, C.; Carvalho, J. J.; Azenha, M. A.; Alpendurada, M. F. *J. Chromatogr., A* **2006**, *1110*, 6-14.
- Vagi, E.; Simandi, B.; Suhada, A.; Hetheylly, U. *Food Res. Intl.* **2004**, *38*, 51-57.
- Yang, K.; Hasenoehrl, E. J.; Griffiths, P. R. *Vibra Spectra* **1997**, *14*, 1-8.
- Ibáñez, E.; López-Sebastián, S.; Ramos, E.; Tabera, J.; Raglero, G. *J. Agric. Food Chem.* **1997**, *45*, 3940-3943.
- Mike, J. A. *Chemometrics in Analytical Spectroscopy*, RSC Analytical Spectroscopy Monograph; The Royal Society of Chemistry: Thomas Graham House, Science Park, Cambridge, 1995.
- Espinosa, S.; Raeissi, S.; Brignole, E. A.; Peters, C. J. *J. Supercrit. Fluids* **2004**, *32*, 63.
- Taylor, L. T. *Supercritical Fluid Extraction: Techniques in Analytical Chemistry*; John Wiley and Sons: New York, 1996; pp 1-11.
- Wang, Z.; Sun, S. Q.; Li, X. B.; Zhou, Q.; Lin, L.; Du, D. G. *Spectros. Spec. Anal.* **2001**, *21*(3), 311-313.
- Esbensen, K. H. *Multivariate Data Analysis-In Prantice*, 5th ed.; CAMO Process AS: Norway, 2002.
- Otto, M. *Pattern recognition and classification, Chemometrics: Statistics and Computer Application in Analytical Chemistry*; Wiley-VCH: Weinheim, Germany, 1999; pp 128-

- 130.
18. Vesper, H.; Nitz, S. *Adv. Food Sci.* **1997**, *19*, 178-183.
19. Ab-Rahman, N. N.; Hassan, M. N.; Ab-Kadir, M. O. *Natl. Prod. Sci.* **1999**, *5*, 75-79.
20. Nik Norulaini, N. A.; Anees, A.; Fatehah, M. O.; Banana, A. A. S.; Zaidul, I. S. M.; Mohd Omar, A. K. *Sep. Purif. Technol.* **2008**, *60*, 272-277.
21. Jaren-Galan, M.; Carmona-Ramon, C.; Minguéz-Mosquera, M. I. *J. Agric. Food Chem.* **1999**, *47*, 2671-2677.
22. Nik Norulaini, N. A.; Anuar, O.; Omar, A. K. M.; AlKarkhi, A. F. M.; Sahena, F.; Zaidul, I. S. M. *Food Bioprod. Process* **2009**, *87*, 152-158.